REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	1998, Dec. 31	FINAL 08/14/94 - 03/31/98

4. TITLE AND SUBTITLE
"Nucleoprotein-Based Nanoscale Fabrication"

5. FUNDING NUMBERS
G: N00014-94-1-1116
PR: 97PR02374-0

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(20-940090)

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10. SPONSORING / MONITORING AGENCY REPORT NUMBER
AGO#: B66018
CAGE#: 5A296

11. SUPPLEMENTARY NOTES
See Attached

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*
See Attached

14. SUBJECT TERMS
DNA (Cytosine-5), Methyltransferases, 5FdC

15. NUMBER OF PAGES
4

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FINAL REPORT

GRANT #: N00014-94-1-1116

PRINCIPAL INVESTIGATOR: Steven S. Smith, Ph.D.

INSTITUTION: City of Hope

GRANT TITLE: Nucleoprotein-Based Nanoscale Fabrication

AWARD PERIOD: 15/August/1994 - 1/April/1998

OBJECTIVE: To develop a series of nanoscale structures and devices based on addressable molecular components.

ACCOMPLISHMENTS: We have provided a clear demonstration of each of the essential scientific principles governing this approach to macromolecular assembly.

First, we have shown that the coupling reaction between methyltransferases and 5-FdC in DNA provides a mechanism for stable attachment of methyltransferases and chimeric methyltransferase fusion proteins in a pre-selected arrangement along a DNA molecule.

In the course of this work we discovered that the FdC dramatically slows the rate of methyltransfer catalyzed by DNA methyltranserases. Slowing of the methyltransferase reaction by FdC could indicate aberrant hydrogen bonding between the fluorine atom on cytosine and groups at the enzyme active site. Alternatively the electron withdrawing power of the fluorine atom may diminish the capacity of the activated intermediate to attack the methyl group on the S-adenosyl-methionine methyl donor. Electronic structure calculations, at the Hartree-Fock Level of theory with the 6-31G* basis set, support the latter possibility. The calculated energy of the Highest Occupied Molecular Orbitals for models of all the possible intermediates at this stage of the reaction is significantly lowered by the presence of the 5-fluorine atom.

Thus the substitution of the targeted pyrimidine ring with fluorine at C5 generates a kinetic barrier to the methyltransfer step in the reaction and a thermodynamic barrier to the beta-elimination step. Either of these barriers can cause the enzyme to stall after it forms a covalent bond with DNA. These findings suggested that other methyltransferase targets that lower the HOMO energy of the catalytic intermediate could be used to target the enzyme to a selected site. When dU is used in place of 5FdC as the base targeted by the enzyme for nucleophilic attack, it formed a mispair with deoxyguanosine (dG). Like 5FdC, dU

created a kinetic barrier to completion of the reaction causing the enzyme to stall. Unlike 5FdC, dU did not create a thermodynamic barrier to beta-elimination and release after methyltransfer; however, the mispaired state of the dU in the dG:dU basepair appears to create a transition-state analog that cannot be easily released by the enzyme.
The data show that dU can be used to substitute for 5FdC in the production of ordered nucleoprotein-based macromolecular assemblies. Thus, the supramolecular chemistry that we have developed for addressable biostructures includes dU as a substitute for FdC.

Second, we have obtained a first demonstration nanoscale addressing. Mobility shift data show that we can decorate a linear DNA molecule with two or three copies of *Hha*I methyltransferase. In these systems *Hha*I methyltransferases were placed along the z axis of the DNA helix so as to produce radial placement of C-termini of the molecules at intervals of π for the test system containing two methyltransferases or $2\pi/3$ for the test system containing three methyltransferases. Coupling occurs with high efficiency (about 20% of input methyltransferase coupled) when the recognition sequences are 35nt apart but only with very low efficiency (about 1% of input methyltransferase coupled) when they are 5nt apart. This is consistent with footprinting experiments suggesting that close placement of sites produces overlap and steric hindrance.

The demonstration of nanoscale addressing with 16-35nt center to center spacing between recognition sites is significant not only because it is the first such observation, but also because it confirms our estimates of footprint size for *Hha*I from kinetic studies. Moreover, obtaining a complex at 5nt center to center spacing even at the expected low yield strongly suggests that these enzymes approach from opposite sides of the helix as expected from the periodicity of B-DNA.

Third, we have demonstrated that methyltransferases with different targeting specificities can be used in the assembly of ordered arrays by constructing an ordered biostructure with M•MspI at a preselected target site and M•HhaI at a preselected target site on the same DNA molecule.

Fourth, we have shown that the properties of a fusion peptide can be altered by the placement of a second protein at an adjacent site. In particular, we observed an enhanced interaction of the M-*Hha*I-dod fusion protein with an anti-body to the dodecamer (dod) fused to the methyltransferase only when it was placed adjacent to M-*Msp*I. This suggests that the oligodeoxynucleotide used to link the two adjacent methyltransferases may adopt a curved conformation in the complex, or that methyltransferase-induced unwinding places

the enzymes in a position to somehow interact in the doubly substituted complex.

CONCLUSION: We have completed a scientific description of the supramolecular chemistry of addressable biostructures.

SIGNIFICANCE: This chemistry provides new approaches to the assembly of biostructures that can serve as new materials, as molecular switches, as components of switching arrays, and as molecular devices capable of performing an ordered operation. Each of these biostructures awaits appropriate engineering based on the scientific principals developed here.

AWARD INFORMATION:

1995-96 Recognized by Burroughs Wellcome Fund and the Federation of American Societies for Experimental Biology as a Wellcome Visiting Professor in Basic Medical Sciences at Oklahoma State University.

1996 Recognized by Department of Biochemistry and Molecular Biology of the University of Oklahoma for Distinguished Contributions in the field of DNA Methylation.

1996 Cover Portrait *Cope* Magazine.

1997 Appointed to Editorial Board of Analytical Biochemistry, Academic Press.

1997 Feynman Award Nominee

1998 Feynman Award Nominee

PATENT INFORMATION: No patents were filed under this proposal.

PUBLICATIONS AND ABSTRACTS (For Total Period of Grant)

1. Laayoun, A. and Smith, S.S. (1995) Methylation of slopped lduplexes, snapbacks and cruciforms by human DNA(cytosien-5)methyltransferase. *Nucleic Acids Research* 23: 1584-1589.

2. Smith, S.S., Niu, L., Baker, D.J., Wendel, J.A., Kane, S. E. and Joy, D.S. (1997) Nanoscale Addressing in Macromolecular Assembly. *Miami Biotechnology Short Reports*, Vol. 8. F.Ahmad *et al.*, eds. IRL at Oxford University Press, p.13.

3. Smith, S.S., Niu, L. Baker, D.J., Wendel, J.A. Kane, S.E.,and Joy, D.S (1997) Nucleoprotein Based Nanoscale Assembly.*Proc. Natl. Acad. Sci. U.S.A.* 94:2162-2167.

4. Wendel, J.A. and Smith, S.S. (1997) Uracil As An Alternatice To 5-fluorcytosine In Addressable Protein Targeting. Abstracts of the 5th Forsight Conference on Molecular Nanotechnology, November 5-8, 1997; Palo Alto, CA

5. Wendel, J.A. and Smith, S.S. (1998) Uracil As An Alternatice To 5-fluorcytosine In Addressable Protein Targeting. Nanotechnology. 8:297-304.